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MEPACRINE-INDUCED SENSITIZATION OF RATS TO BACTERIAL ENDOTOXIN

S. L. Gartner and K. M. M. Shakir

Naval Medical Research
and Development Command
Bethesda, Maryland 20814-5044

Department of the Navy
Naval Medical Command
Washington, D.C. 20372-5210

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The experiments reported herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

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The experiments conducted herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Resources, National Research Council.

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INTRODUCTION

Investigations in the past several years have implicated prostaglandin release in the pathogenesis of endotoxin shock (4, 5). These studies have focused on the observation that prostaglandin levels are increased following endotoxin administration. Furthermore, indomethacin, a compound that inhibits prostaglandin formation (6), has been shown to protect animals from bacterial endotoxin (12). Since another effect of indomethacin is to inhibit phospholipase A_2 (10, 11), the initial purpose of this study was to determine whether or not other inhibitors of phospholipase A_2, such as mepacrine, would also be protective. Information of this nature would be useful in understanding the mechanism of endotoxin toxicity, as well as a possible treatment for sepsis. Thus, we injected rats with endotoxin and mepacrine. Instead of protection, however, we found that mepacrine sensitized the animals to endotoxin. A preliminary account of this work has been presented in abstract form (7).

METHODS

Mepacrine was a gift from the Sterling-Winthrop Research Institute, Rensselaer, New York. S. enteriditis endotoxin (Boivin preparation lot #678167) was purchased from Difco Laboratories, Detroit, Michigan. Radiolabelled chemicals; leucine ^{14}C (56.9 mCi/mmole), thymidine ^{14}C (47.5 mCi/mmole), and uridine ^{3}H (25.4 Ci/mmole) were obtained from New England Nuclear, Boston, Massachusetts.

Lethality studies were performed on adult, male Sprague-Dawley rats weighing between 180 and 220 grams. Compounds were injected separately in a volume of approximately 0.5 ml while the rats were under ether anesthesia. Various doses of the S. enteriditis endotoxin were dissolved in phosphate buffered saline (PBS) and administered via the femoral vein. Immediately following the endotoxin, animals received intraperitoneal injection of either PBS (control) or mepacrine (50 mg/Kg) dissolved in PBS. Mortality was observed after 72 hours and the data were analyzed for statistical significance by the maximum likelihood method (9).

For the studies on protein and nucleic acid synthesis, spleens were removed from rats and the cells were gently teased from the spleen in minimal essential media containing 25 mM N-2-hydroxethylpiperazine N-2-ethanesulfonic acid (HEPES) and 1% fetal calf serum. The splenocytes were depleted of red blood cells by treatment with ammonium chloride lysing buffer, washed, and passed through nylon (HC3-110, Tetco, Inc., Elmsford, NY). Splenocytes (approximately 1 x 10^7 cells/ml) were incubated at 37°C in 3 ml of minimal essential media containing 25 mM HEPES and 1% fetal calf serum. After a 10-minute preincubation period, 10 uCi of radiolabelled leucine, uridine, or thymidine were added to start the reaction. Aliquots (0.5 ml) were removed at timed intervals and incorporation of radioactivity into protein or nucleic acid was measured by precipitation with 0.5 ml of 20% trichloracetic acid. The precipitates were washed three times dissolved in 1 M KOH and neutralized with 1 N HCl. Radioactivity was determined by liquid scintillation spectrometry using Aquasol-2.

RESULTS

The results in Table 1 show that mepacrine (50 mg/Kg) increased the sensitivity of rats to bacterial endotoxin approximately threefold from 15.9 to 4.6 mg endotoxin/Kg. The dose of mepacrine (50 mg/kg) that was used was not toxic when administered without endotoxin (16 rats). Furthermore, intraperitoneal injection of mepacrine (100 mg/Kg, N=16), followed by intravenous PBS produced no deaths. These experiments clearly show that mepacrine, instead of being protective, actually produced significant sensitization ($p<0.0001$) to endotoxin.

In addition to the inhibition of phospholipase A_2 activity, mepacrine has other effects that may be responsible for the increased sensitization to bacterial endotoxin. One of the other actions of mepacrine is the inhibition of protein and nucleic acid synthesis (13), and since other agents that inhibit protein and nucleic acid biosynthesis are known to sensitize animals to endotoxin (1), we examined the effect of mepacrine on the ability of rat splenocytes to synthesize protein and nucleic acid. These data in Figure 1 show that 10^{-4} M mepacrine markedly inhibited protein and nucleic acid synthesis: leucine incorporation into protein was reduced to 11% of control; uridine incorporation into RNA was reduced to 1% of control and thymidine incorporation into DNA was reduced to 20% of control in the presence of 10^{-4} M mepacrine. Assuming that 50 mg mepacrine/Kg dose used in the lethality studies were distributed evenly throughout the body of the rat, it would correspond very closely to the 10^{-3} M mepacrine concentration that produced these marked effects on spleen cells <u>in vitro</u>.

TABLE 1. EFFECT OF MEPACRINE ON LETHALITY OF ENDOTOXIN IN RATS

Treatment	LD_{50} (mg/Kg)	Significance
vehicle + endotoxin (70 rats)	15.9	
mepacrine (50 mg/Kg) + endotoxin	4.6	< 0.0001

Rats were given intravenous injection of endotoxin and either vehicle or mepacrine as described in the Methods section. Mortality was observed after 72 hours.

Effect Of Mepacrine On Protein And Nucleic Acid Synthesis In Rat Splenocytes

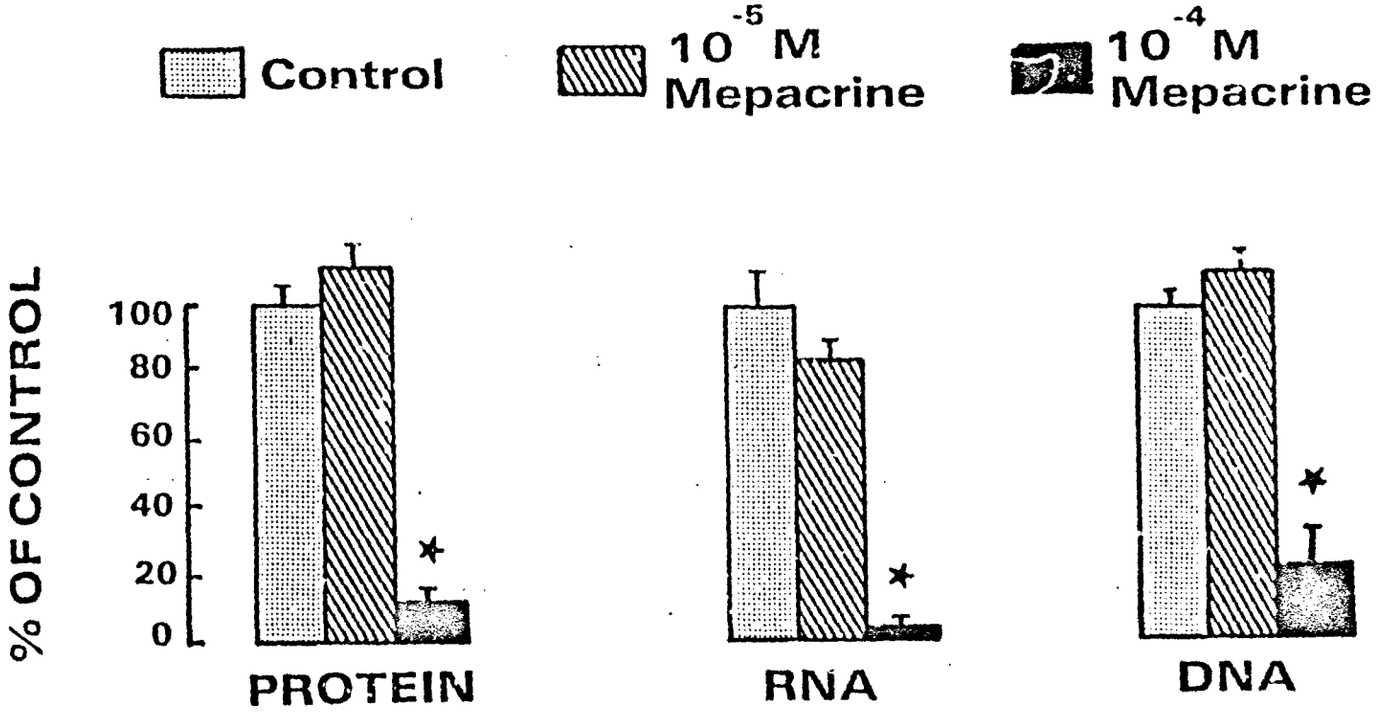

FIGURE 1

DISCUSSION

Mepacrine (Quinacrine, Atabrine ^{R}) is an acridine derivative developed in the 1930s for treatment of malaria. Since the development of more effective, less toxic antimalarials, mepacrine is no longer used for this purpose. When taken orally, mepacrine is readily absorbed and accumulates predominantly in liver and spleen. Intracellularly, it is distributed in the nucleus and lysosomal fractions (13). Besides its action as an inhibitor of phospholipase A_2 (14), mepacrine has several other biological activities. These include inhibition of glucose metabolism and nucleic acid synthesis (13), inducer of interferon (8) and inhibition of calmodulin-activated Ca-ATPase and phosphodiesterase (16). Like other acridine dyes, mepacrine forms a tight complex with DNA which effectively blocks DNA-dependent DNA and RNA polymerase reactions (3). This later effect of mepacrine probably explains the marked depression of protein and nucleic acid synthesis that we observed in rat splenocytes. The inhibitory effect of mepacrine on phospholipase A_2 activity has been well documented (14, 15). For example, Vadas' work shows that mepacrine (10^{-6} to 10^{-3} M) inhibits phospholipase A_2 in rabbit peritoneal exudate cells (14). However, the data of Chan et al. (2), using rat platelets, suggests there might be a biphasic modulation of phospholipase A_2 activity depending upon the concentration of mepacrine. At low mepacrine concentrations (50 to 100 uM) inhibition of phospholipase A_2 occurs, while at a higher mepacrine concentration (500 uM) there is stimulation of activity (2). This brief description of the diverse effects of mepacrine suggests that this drug could have multiple actions that effect survival after endotoxin administration.

In the present study, we found that a nontoxic dose of mepacrine sensitizes rats approximately threefold to bacterial endotoxin. The mechanism of the increased sensitivity caused by mepacrine cannot be determined from our current data, but the fact that mepacrine did effectively inhibit protein and nucleic acid synthesis and that other agents that inhibit protein and nucleic acid synthesis also sensitize to endotoxin suggests that mepacrine may also be acting in this way. Our data on the mepacrine-endotoxin interaction does illustrate the complexity of using whole animals models, as well as the danger of viewing one action of a drug, such as phospholipase A_2 inhibition, and neglecting side effects that may produce very opposing results.

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FIGURE LEGEND

Figure 1. Protein, RNA, and DNA synthesis were measured by incubating splenocytes with radiolabelled leucine, uridine, or thymidine, and counting the trichloroacetic acid precipitates as described in Methods section. Experiments were performed at least three times and the results shown are that of one experiment with individual assays performed in triplicate. The data are expressed as % of control incorporation + S.D. Statistical analysis was performed using the Student's t test, *$p<0.011$.